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GENETIC POLYMORPHISM OF BUFFALO *BUBALUS BUBALIS BUBALIS* BY CYTOGENETIC AND MOLECULAR MARKERS

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Aim. To estimate genetic polymorphism of buffaloes *Bubalus bubalis bubalis* by the characteristics of karyotype and microsatellite loci of DNA. **Methods.** The method of preparing chromosome preparations using blood lymphocytes of 30 animals and the analysis of 9 microsatellite loci of DNA, recommended by the International Society for Animal Genetics (ISAG) for cattle genotyping, were used in the investigation. **Results.** Based on the results of the analysis of GTG–stained preparations of metaphase chromosomes, it was determined that the karyotype of the investigated animals consisted of 25 pairs of chromosomes, which identified them as river (murrh) buffaloes (*Bubalus bubalis bubalis*). The average frequency of detected quantitative and structural chromosome aberrations in the buffalo population under investigation was 13.58 ± 3.18 % for males and 14.8 ± 2.88 % for females. Active centers of nucleolus organizers were found on six pairs of chromosomes: 3p, 4p, 6q, 21q, 23q and 24q. In nine investigated microsatellite loci of DNA, 61 allele variants were identified with the variation from four alleles (ILST 006) to 11 (TGLA) with the average number of alleles per locus being 6.77. The expected heterozygosity (He) exceeded the average value of the observed heterozygosity (Ho) which demonstrated the use of inbreeding in the breeding of the investigated group of animals. **Conclusions.** The application of quantitative and morphological characteristics of the karyotype and microsatellite DNA–markers (ILST005 (ILSTS005), ILST006 (D7S8), BM1818 (D23S21), BM2113 (D2S26), ETH10 (D5S3), ETH225 (D9S1), SPS115(D15), TGLA126 (D20S1), TGLA122 (D21S6) demonstrated their informative value and reasonability of their use for genetic monitoring of buffaloes in Ukraine with the purpose of developing selection programs of keeping and breeding this species.

Key words: buffaloes *Bubalus bubalis bubalis*, genetic polymorphism, chromosomes, aberrations, microsatellite loci of DNA.

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INTRODUCTION

In recent decades, the animal breeding of Ukraine has been going through the crisis, manifested by reduction in the cattle population. The development of animal breeding programmes, non-traditional for our country, to satisfy human needs for food products is becoming more urgent.

From this standpoint, attention is drawn to the breeding of buffaloes *Bubalus bubalis*, the population of

which demonstrates stable increase in South Europe, the USA, Canada, Israel, where a new animal breeding programmes is being formed – buffalo breeding (Guzeev *et al.*, 2016).

The uniqueness of this introduced species of animals for countries with no previous tradition of breeding it lies in high quality of the obtained products, dairy products, first and foremost. Dairy productivity of buffaloes is not high – 1500–2500 l of milk with the fat content of 7.6–10.5 %, protein content of 4.3–5.1 %

(Guzeev Yu 2014), but its value is hard to overestimate: the milk of buffalo cows has twice more fat and protein, almost 60 % more minerals, including calcium and vitamins compared to the milk of cows. At the same time, it has less calories and no casein, thus it is considered to be dietary. Whole buffalo milk is the material for the production of valuable high quality gourmet cheese *Mozzarella di Bufala Campana*, which meets the requirements of the Consortium for the Protection, the Protected Designation of Origin (DOP), guaranteed by the Ministry of Agriculture of Italy.

Buffaloes are adapted to high temperatures of the environment which is especially relevant in conditions of the global climate changes. Due to strong immunity, these animals also have higher resistance to infections and parasites, spread in the zones of hot humid climate as well as to tuberculosis and foot-and-mouth disease, piroplasmosis, anaplasmosis, foot rot, mastitis and other diseases, wide-spread among cattle, and they do not require vaccination (Guzeev Yu 2012).

In addition, river buffaloes may fulfill a purely ecological function, improving the state of the ecosystem as natural meliorators on waterlogged land, which is of low use for agriculture. Buffalo breeding may also promote business for shepherds, guards, cheese producers. Taking into consideration the fact that the production of buffalo milk is cheaper as compared to that of cow milk, as buffaloes mainly consume hard and fibrous feeds, and do not require considerable energy losses in the process of technological keeping and breeding (Guzeev Yu, Vinnychuk D 2015), the perspectives of buffalo breeding in modern Ukraine become obvious.

100 years ago there were over a thousand buffaloes of Asian origin, *Bubalus bubalis bubalis*, in Ukrainian Carpathian mountains, used as animal traction, a source of meat, milk, skin, bones and manure. The latter was used in the building of houses and heating them, improving soil fertility, etc. (Guzeev Yu 2014).

At present there are about 120 buffaloes in Ukraine, and they are bred for the production of milk and meat (Guzeev Yu 2012).

However, the buffalo is still commercially underestimated as a species of agricultural animals.

The selective, productive and reproductive traits of buffaloes have already been studied and described by many researchers. The assessment of the impact of different environmental factors on the level of milk yield and quality has been conducted (Guzeev Yu 2012, Gu-

zeev Yu, Vinnychuk D 2015). The genetic forecast, made on the basis of buffalo genealogy, can be confirmed now using cytogenetic (Dawood M *et al.* 2014; Ali A 2012) and molecular markers (Talib A *et al.* 2014; Guzeev Yu 2016), but the communications about conducting such studies are extremely rare.

Based on the results of the study on karyotypes of *Bubalus bubalis*, Professor Bondoc of UPLB (University of the Philippines Los Banos) *et al.* (Bondoc O L *et al.* 2002) determined the correlation between the karyotype characteristics and some productivity traits of animals, and made a conclusion that karyotype characteristics may be used as relevant criteria in selecting potentially productive young water buffaloes.

The scientists of Universiti Putra Malaysia (Nor' Ammar Liyana Shaari *et al.* 2019) state that the karyotype analysis may be used to differentiate between water (*Bubalus bubalis*) and river (or murrh, *Bubalus bubalis bubalis*) buffaloes and their hybrids. However, the systemic cytogenetic study on buffaloes is yet to be conducted, which means that most chromosomal aberrations are to be detected (Iannuzzi L *et al.*, 2014).

Thus, complex genetic studies of buffaloes, involving the polymorphism analysis of chromosome set and microsatellite DNA-loci may be used in future to elaborate rational strategies of breeding and preserving buffaloes (Kumar S 2006).

The aim of our present research was to study genetic polymorphism of the Ukrainian population of river (murrh) *Bubalus bubalis bubalis* by the characteristics of karyotype and microsatellite loci of DNA.

MATERIALS AND METHODS

The genetic study was conducted using 30 phenotypically normal reproductive river buffaloes – 9 males and 21 females, aged from 1 to 5 years old, with the live bodyweight of 450–600 kg, black, kept at Holoziivo LLC in the village of Hoholiv in Kyiv region. The samples of biological material (venous blood and retracted ear tissue) were used as the genetic study material.

The cytogenetic studies were conducted in 2017–2018 at the Genetics Laboratory of the M. V. Zubets Institute of Animal Breeding and Genetics, NAAS. The molecular studies were conducted at the Laboratory of Molecular Genetics and Cytogenetics of Animals, the Center of Biotechnology and Molecular Diagnostics of the State Scientific Institution All–Russia Animal Breeding Institute with the participation of Yu. V. Guzeev.

The culture of lymphocytes and chromosome preparations were conducted by the method of *Moorhead et al. (1960)*. According to this method, 5 ml of the cultural medium RPMI 1640 (Sigma, USA), supplemented with 20 % of fetal bovine serum and 0.1 ml of phytohemagglutinin (FGA, Sigma, USA), were added to 1 ml of peripheral buffalo blood and cultivated for 48 h at +37 °C. Two hours prior to the completion of cultivation, 0.05 % colchicine solution was introduced (Serva, Germany). The precipitate of cells after centrifugation was processed with the hypotonic solution of KCl (0.75 M) for 20 min at 37 °C. Finally, the material was fixed and washed with methanol-acetic fixator (3 : 1).

35–40 metaphases, stained with Giemsa mixture (Merk, Germany), were analyzed for each animal using a Carl Zeiss binocular microscope (Germany); the diploid numbers of chromosome sets and chromosome morphology were determined for each specific karyotype according to the standard karyotype of river buffaloes (Iannuzzi L. 1994). The representative metaphases were registered using a digital camera. Some metaphases were used for staining by Ag–NOR method to detect nucleolus organizer regions (NOR) in metaphase chromosomes. The staining procedure was conducted as follows: a drop of 0.2 % solution of formic acid (pH 2.6–2.7) and 50 % solution of AgNO₃ (in 1 : 1 ratio) was applied to the specimen slide. The preparation was placed in a Petri dish on moistened filtration paper and kept in the thermostat for 5–8 min at 620 °C. NOR were detected on telomeres of the corresponding chromosomes as dark spots.

The molecular-genetic analysis was conducted by 9 microsatellite loci of DNA (ILST005 (ILSTS005), ILST006 (D7S8), BM1818 (D23S21), BM2113 (D2S26), ETH10 (D5S3), ETH225 (D9S1), SPS115(D15), TGLA126 (D20S1), TGLA122 (D21S6), recommended by the International Society for Animal Genetics (ISAG) for cattle genotyping (32nd Conference of ISAG 2010).

DNA isolation was conducted using a set of reagents for DNA isolation, DNA-sorb-B (AmpliSens, RF) according to the manufacturer's instructions.

The amplification in PCR reaction was conducted in the reaction mixture of 15 µl, consisting of 2.5 µl 10 × PCR-buffer, 1.5 µl of the mixture of dNTP, 0.3 µl Taq-polymerase, 3 µl of the mixture of primers, 6.2 µl of deionized water and 2 µl of DNA.

The polymerase chain reaction was conducted using Veriti 96-Well thermal cycler (Applied Biosystems,

USA) with the following parameters: 1 stage – initial denaturation (95 °C – 10 min), 2 stage – 30 cycles (95 °C – 30 sec, 60 °C – 30 sec, 72 °C – 1 min), final – 10 min at 72 °C. The capillary electrophoresis of amplification products was conducted at MegaBace500 device.

The studies involved the determination of the number of alleles, the number of effective allelerational observed (stimated expected (He) heterozygosity, polymorphic information content (PIC), fixation index (Fis). MegaBace Genetic Profiler 2.0 was used to determine the size of alleles. Statistical processing of the data was conducted in PowerStatsV12 (Promega), GENALEX 6 (Paetkau D *et al.* 2004).

RESULTS OF INVESTIGATIONS

The cytogenetic analysis of GTG-stained preparations of metaphase chromosomes demonstrated that the karyotype of the investigated buffaloes consisted of 25 pairs of chromosomes: in females – 2n = 50 (XX), in males – 2n = 50 (XY), which identified them as river buffaloes, as the karyotype of water buffaloes had 24 pairs of chromosomes (2n = 48). This cytogenetic difference in the number of chromosomes could result from the association of chromosomes 4 and 9 which had occurred in the karyotype of water buffaloes at some evolutionary stage (Degrandi T *et al.* 2014). The obtained results of analyzing the chromosome set of Ukrainian buffaloes are completely homologous to the standard karyotype of buffaloes (Iannuzzi L 1994), covering 5 pairs of autosomes of metacentric structure (from the second to the fifth) and 19 pairs of acrocentric structure (from the sixth to the twenty-fourth), whose sizes gradually become shorter. As for sex chromosomes: X is the largest and Y – the smallest acrocentric chromosomes (Figure). Similar results in terms of the number and structure of chromosomes in the karyotype of river buffaloes were reported by other researchers (Alikhani J *et al.* 2018).

The cytogenetic investigation established that the average frequency of detected quantitative and structural chromosome aberrations in the buffalo population under investigation was 13.58 ± 3.18 % for males and 14.8 ± 2.88 % for females with statistically insignificant difference. The analysis of chromosome sets revealed quantitative disorders in the karyotype, which were presented with aneuploidy and polyploidy in the spectrum of registered aberrations and occurred with the frequency of 9.18 % for males and 9.8 % for females. The results of cytogenetic investigation of buffaloes in Egypt were reported by Ahmed *et al.* (Ahmed,

S *et al.*, 1998), who noted that in their investigation the total level of chromosome aberrations was 7.6 ± 1.6 %. In another investigation (Ahmed, S 2005), the level of aberrant cells was up to 37.6 % which was much higher as compared to our results.

In addition, we have detected structural chromosomal abnormalities in some buffaloes, but they had no impact on the phenotype of these animals and did not result in their barrenness. The most common chromosomal rearrangements were found to be breakage, single and paired fragments.

A number of identified structural chromosomal aberrations, including deletion, breakage, fragmentation, ring formation, and central fusion of chromosomes were found in the study of Egyptian buffaloes by Rushdi *et al.* (Rushdi H *et al.*, 2017.) The frequency of polyploid and aneuploid buffalo cells in their investigation was 9.6, 1.2 % respectively.

No severe chromosomal pathology was found in any animal. However, one buffalo cow had fragile sites in some autosomes with the frequency of 3.0 %, which are visually indicated as areas of uncolored spaces. It is known that such fragile chromosomal sites are specific loci with gaps or translocations in metaphase chromosomes, inclined to having impulsive breakage which may impact the structural integrity of genes (Durkin S, Glover T 2007).

To ensure more precise analysis of the chromosomal set and identification of specific chromosome sites of Ukrainian buffaloes, we investigated NORs in chromosomes, the number and activity of which, according to scientific literature, reflected potential capabilities of cells to synthesize proteins and demonstrate the functional state of cells and the organism of animals as a whole. The differentiated staining of chromosomes Ag-NOR was used to detect active areas of nucleolus organizers on six pairs of chromosomes: on short arms of two pairs (3p, 4p) and on long arms of four pairs of chromosomes (6q, 21q, 23q and 24q). Similar data were published by Degrandi *et al.* (Bernard *et al.*, 2014). The study of metaphase plates of the investigated animals revealed from 1 to 12 NORs per cell, the average number being 2.82.

The possibility of accurate identification of chromosomes, containing nucleolus organizers, is a relevant moment for the comparison of mammals of Bovidae species by karyotype characteristics. As per Italian scientists (Iannuzzi L, *et al.* 2014), NOR-banding demonstrated that chromosome 6 of river buffalo was homologous to chro-

somosome 3 of cattle (Di Berardino D *et al.* 2014) and to chromosome 1p of sheep (Di Meo G *et al.* 1991).

The investigation of microsatellite DNA-loci of Ukrainian buffaloes revealed 61 allele variants in nine investigated loci with the variation from 4 (ILST 006) to 11 (TGLA). The average number of alleles per locus was 6.77 (Table 2).

The average number of effective alleles (Ne) per sampling was 4.0, the highest Ne value was registered for locus BM 1818 (5.0) and the lowest (2.0) – for SPS 115.

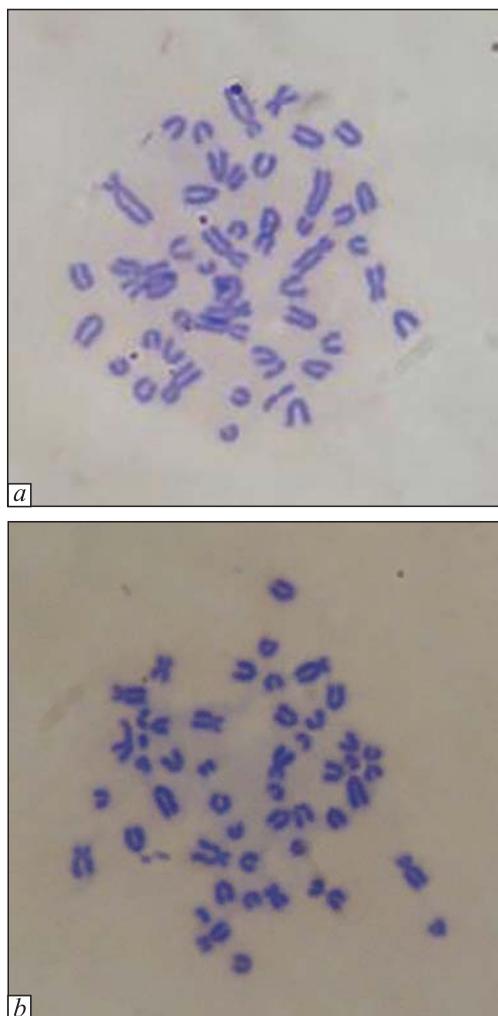
One of the key parameters, used to analyze genetic polymorphism of the population, is its heterozygosity. It is especially relevant for our investigation because almost two decades of buffalo breeding in Holoziivo LLC resulted in an urgent problem of reproducing the population due to compelled inbreeding. A common ancestor for most animals is a third-generation sperm giver.

The use of inbreeding in the investigated buffalo population is confirmed by the excess of homozygous genotypes: on average, the expected heterozygosity of 0.045 prevailed over the average value of observed heterozygosity. The excess of homozygous genotypes was registered in more than a half of investigated loci, with the highest value being for locus TGLA126.

The results of investigations demonstrated that the level of observed heterozygosity (Ho) varied from 0.355 for locus BM 2113 to 0.690 for BM 1818 with the average value of 0.540, here the indices of expected

Table 1. The spontaneous frequency of chromosomal aberrations in buffalo blood lymphocytes

Chromosomal aberrations	Frequency of chromosomal aberrations per 100 cells	
	Males (n = 9)	Females (n = 21)
Genomic:		
Aneuploidy	8.64 ± 1.20	8.98 ± 1.16
Polyploidy	0.54 ± 0.03	0.82 ± 0.10
Structural:		
Chromosomal breakage and fragments	4.40 ± 0.06	4.85 ± 0.09
translocations	0.85 ± 0.09	0.15 ± 0.02
Total of aberrant cells	13.58 ± 3.18	14.8 ± 2.88
Total of analyzed cells	450	735



The metaphase plates of a river buffalo at norm: a – male, $2n = 50, XY$; b – female $2n = 50, XX$, magnification: field lens $\times 100$; ocular lens $\times 10$

heterozygosity (H_e) fluctuated from 0.240 for locus TGLA 122 to 0.800 for locus TGLA 126.

The average values of F_{is} were positive and had significant deviations from zero which demonstrated the selection pressure on the population of animals. The highest F_{is} value was found for microsatellite loci SPS115 and TGLA126.

The value of PIC, calculated to assess the informative value of microsatellite DNA-loci, varied from 0.250 to 0.670.

According to Bishop and Kappes (Bishop M. and Kappes S. 1994), the loci with PIC value over 0,500 are considered highly polymorphic, the loci with PIC within 0.250–0.500 are moderately polymorphic and if $PIC < 0.250$, then markers are of low polymorphism. As for the loci investigated, the buffalo micropopulation was found to be moderately polymorphic ($PIC = 0.490$). The exception was found in loci ILST 005, ILST 006, BM 1818 and TGLA 1263, whose PIC value exceeded the average one.

The investigation of Indian water buffaloes by 108 pairs of microsatellite primers, presented by Navani *et al.* (Navani N *et al.* 2002) established that average polymorphism index of loci was 0.66 ± 0.02 , and the average number of alleles per one polymorphic locus was 4.50 ± 0.20 . These indices are somewhat higher from similar ones, received in our investigations.

Elbeltagy *et al.* investigated 104 Egyptian and Italian buffaloes by 15 pairs of microsatellite primers, used to study the genome of cattle. The investigation findings demonstrated that the observed level of heterozygosity

Table 2. The heterozygosity, polymorphism degree of microsatellite markers of river buffaloes in Ukrainian population

Locus	Number of alleles (N_a)	Number of effective alleles (N_e)	Range of allele lengths, bp	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Fixation index (F_{is})	Polymorphism information content of the locus (PIC)
cILST 005	6	5	176–193	0.570	0.550	–0.036	0.530
ILST 006	8	4	289–305	0.520	0.600	0.130	0.560
BM 1818	6	5	256–280	0.690	0.770	0.103	0.600
BM 2113	4	3	234–242	0.355	0.310	–0.145	0.267
ETN 10	6	4	140–164	0.400	0.281	–0.423	0.380
ETN 225	9	4	248–264	0.480	0.620	0.225	0.610
SPS 115	5	2	71–99	0.420	0.600	0.300	0.250
TGLA 126	6	4	136–182	0.565	0.800	0.294	0.670
TGLA 122	11	4	248–264	0.380	0.240	–0.583	0.570
Average value	6.77	4.0		0.540	0.585	0.076	0.490

for Egyptian buffalo was 71 %, that for Italian buffalo – 65 % and these indices were a quarter higher than the index of observed heterozygosity of buffaloes in our study (Elbeltagy *et al.* 2008).

Indian scientists Mishra *et al.* (Mishra *et al.* 2009) reported the cytogenetic and molecular analysis of the buffalo population in Eastern India, in which they determined a number of chromosomes in the karyotype, notable for river buffaloes – $2n = 50$, and the study of 25 microsatellite loci in the DNA structure demonstrated a moderate level of genetic diversity with the excess of homozygous genotypes. The value of Fis was positive, similar to our investigation, but the value for homogeneity level was much higher (0.178 against 0.076). Having analyzed the data obtained, Mishra *et al.* (Mishra *et al.* 2009) came to the conclusion about the reasonability of using the multiplex panel of markers, recommended for genetic analysis of the origin of cattle and assumed that this set of microsatellite markers may be used in a test for the validity of the origin of buffaloes as well. This is also confirmed by Coletta *et al.* (Coletta *et al.* 2007) and Vieira (Vieira 2011), who reported that this panel had already been used to investigate Mediterranean buffaloes in Italy and buffaloes in Brazil. However, they recommend that the set of microsatellite markers should be expanded for genetic studies.

CONCLUSIONS

The complex investigation of cytogenetic and molecular characteristics of river buffaloes, bred in Ukraine, determined genetic polymorphism both on the chromosomal and molecular levels.

The analysis of the karyotype of investigated buffaloes determined the number of aberrant cells per 100 examined males to be 13.58 ± 3.18 , and that for females – 14.8 ± 2.88 . Quantitative and structural changes in the chromosome set demonstrate the need for cytogenetic monitoring of buffaloes with the purpose of detecting hereditary chromosomal abnormalities and determining the reasons of their chromosomal instability. The conducted analysis of the karyotype of the buffalo group in Ukraine demonstrates that according to the diploid number of chromosomes ($2n = 50$) they belong to river buffaloes (murrh) *Bubalus bubalis bubalis*.

The obtained results of the application of microsatellite loci, recommended by the International Society for Animal Genetics for genetic analysis of cattle (ILST005, ILST006, BM1818, ETN225, TGLA126, TGLA122), give grounds for the use of the abovementioned

panel of microsatellite DNA–markers in genetic monitoring of buffaloes and the application of the obtained information for the elaboration of selection programs in breeding these animals in Ukraine.

Adherence to ethical principles. All the international, national and institutional principles of caring for animals and using them have been complied with.

Conflict of interests. The authors declare the absence of any conflicts of interests.

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Генетичний поліморфізм буйволів *Bubalus bubalis bubalis* за цитогенетичними і молекулярними маркерами

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Мета. Дослідити генетичний поліморфізм буйволів *Bubalus bubalis bubalis* за характеристиками каріотипу і мікросателітними локусами ДНК. **Методи.** Для дослідження використовували метод приготування препаратів хромосом із лімфоцитів крові 30 тварин і аналіз 9 мікросателітних локусів ДНК (ILST005 (ILSTS005), ILST006 (D7S8), BM1818 (D23S21), BM2113 (D2S26), ETH10 (D5S3), ETH225 (D9S1), SPS115(D15), TGLA126 (D20S1), TGLA122 (D21S6), які рекомендовані International Society Animal Genetics (ISAG) для генотипування великої рогатої худоби. **Результати.** За результатами аналізу GTG-забарвлених препаратів метафазних хромосом встановлено, що каріотип досліджених тварин складається із 25 пар хромосом, що ідентифікує їх як азійських буйволів (*Bubalus bubalis bubalis*) або буйволів річкових (*river buffalo*). Середня частота виявлених числових і структурних хромосомних аберацій у буйволів дослідженої популяції складає $13,58 \pm 3,18$ % у самців і $14,8 \pm 2,88$ % у самок. Виявлено активні райони ядерцевих організаторів на шести парах хромосом: 3р, 4р, 6р, 21q, 23q і 24q. У дев'яти досліджених мікросателітних локусах ДНК ідентифіковано 61 алельний варіант з варіюванням від 4 алелів (ILST 006) до 11 (TGLA) із

середнім числом алелів на локус 6,77. Теоретично очікувана гетерозиготність (He) переважала середнє значення фактичної гетерозиготності (Ho), що свідчить про використання інбридингу у розведенні дослідженої групи тварин. **Висновки.** Використання числових і морфологічних характеристик каріотипу і мікросателітних ДНК-маркерів свідчить про їх інформативність і доцільність застосування для генетичного моніторингу буйволів в Україні з метою розроблення селекційних програм із збереження і розведення даного виду тварин.

Ключові слова: буйволи *Bubalus bubalis bubalis*, генетичний поліморфізм, хромосоми, аберації, мікросателітні локуси ДНК.

**Генетический полиморфизм буйволів
Bubalus bubalis bubalis по цитогенетическим
и молекулярным маркерам**

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Цель. Исследовать генетический полиморфизм буйволів *Bubalus bubalis bubalis* по характеристикам каріотипа і мікросателітним локусам ДНК. **Методи.** Для исследования использовали метод приготовления препаратов хромосом из лимфоцитов крови 30 животных и анализ 9 микросателітних локусов ДНК (ILST005 (ILSTS005), ILST006 (D7S8), BM1818 (D23S21), BM2113 (D2S26), ETH10 (D5S3), ETH225 (D9S1), SPS115(D15), TGLA126 (D20S1), TGLA122 (D21S6), которые рекомендованы International Society Animal Genetics (ISAG) для генотипирования крупного рогатого скота. **Результаты.** По результатам анализа GTG-окрашенных препаратов метафазных хромосом установлено, что каріотип исследованных животных состоит из 25 пар хромосом, что идентифицирует их как доместіцированную форму азиатских буйволів *Bubalus bubalis bubalis* речного типа или буйволів речных (*river buffalo*). Средняя частота выявленных числовых и структурных хромосомных аберацій у буйволів исследованной популяції составляет $13,5 \pm 3,18$ % у самцов и $14,8 \pm 2,88$ % у самок. Выявлены активные районы ядрышковых организаторов на шести парах хромосом: 3p, 4p, 6q, 21q, 23q и 24q. По девяти исследованным

микросателітним локусам ДНК идентифіцировано 61 аллельный вариант с варьированием от 4 аллелей (ILST 006) до 11 (TGLA) со средним числом аллелей на локус 6,77. Теоретически ожидаемая гетерозиготность (He) преобладала над средним значением фактической гетерозиготности (Ho), что свидетельствует об использовании инбридинга в разведении исследованной группы животных. **Выводы.** Использование числовых и морфологических характеристик каріотипа и микросателітних ДНК-маркерів свидетельствует об их информативности и целесообразности применения для генетического мониторинга буйволів в Украине с целью разработки селекционных программ по сохранению и разведению данного вида животных.

Ключевые слова: буйволи *Bubalus bubalis bubalis*, генетический полиморфизм, хромосоми, аберації, микросателітні локуси ДНК.

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